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14. ABSTRACT
Four different diatom species were cultured and cell wall silica isolated from them. Silicified structures were examined by scanning electron microscopy. The overall cell dimensions were determined, and the dimension of fine pores in the wall. Two species had regularly repeating pore structures that potentially could generate photonic band gap phenomenon. The absorbance and fluorescence characteristics of silica from each species were determined. Absorbance increased with decreasing wavelength, and fluorescence from native diatom silica was low. The ability of different dyes to stain silica in living diatoms was investigated. Out of 6 previously untested dyes, two, rhodamine B and rhodamine 6G, stained diatom silica. Rhodamine 123 was previously shown to stain diatom silica, and we showed that fluorescence of this dye survived the harsh acid treatment required to isolate the silica in pure form. Attempts to measure photonic band gap phenomenon from the silica were unsuccessful; a high degree of scattering in the sample prevented this.

15. SUBJECT TERMS

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Final Report

Investigation into the Optical Properties of Nanostructured Silica from Diatoms

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Grant No. F49620-01-1-0165

Objectives

The overall objective of this project was to investigate the optical properties of silica isolated from the unicellular algae known as diatoms. **Specific objectives** were to:

1. Grow different diatom species and prepare purified silica.
2. Examine this material by electron microscopy (EM) to determine overall shape and fine pore structure characteristics.
3. Determine absorbance and fluorescence characteristics of the purified silica.
4. Test a variety of dyes for ability to stain diatom silica *in vivo*, and determine whether dye treatment survived the harsh acid treatment used for silica purification.
5. Determine whether material from any species had photonic band gap properties.

Status of Effort

Items 1-4 in the specific objectives have been completed. Item 5 was attempted, but a technical problem (described below) prevented successful measurement of photonic band gap properties.

Accomplishments/New Findings

1. Growth of Species and Silica Purification.

The diatom species used in this study were *Cylindrotheca fusiformis, Cyclotella meneghiana, Navicula pelliculosa,* and *Nitzschia alba*. Cultures of each were grown to high cell density in artificial sea water medium (Darley and Volcani 1969), harvested, and cell wall silica was extracted by repeated hot sulfuric/nitric acid treatment (Reimann et al. 1965) until the material was colorless. The silica was washed in 1 M Tris-HCl, pH 7.5, and twice in water prior to drying under vacuum.

2. Electron Microscopic Examination to Determine Overall Shape and Fine Pore Structure.

Silicified structures were examined by SEM. SEM images of *C. fusiformis* are not presented because silica isolated from this species only consists of two classes of thin silicified strips (Fig. 1A), however a representation of this species is given in Fig. 1B. *C. meneghiana* is a centric diatom (radially symmetrical) with a solid silica surface except for fields of pores arranged on ridges on the outer portion of the frustule (another term for the silicified cell wall in diatoms), as shown in Fig. 1 C and D. This species was about 12 μm across. Pores were

irregularly shaped and sized (Fig. 1E), ranging from 17-240 nm across. *Navicula pelliculosa* has been described previously (Reimann et al. 1966), and cells in this study were on the order of 7 μm long and 4 μm wide (Fig. 1F). Pore sizes varied in the two examples shown; in Fig. 1F pores were smaller (ca. 100 nm) whereas in Fig. 1G pores were 150-175x130-260 nm. Possible reasons for these differences are the rate of growth at different stages of the culture or the availability of silicate, both factors that can alter the extent of silicification (Martin-Jézéquel, et al., 2000). *Nitzschia alba* was on the order of 25 μm long and 3 μm wide (Fig. 1H), and had regularly repeating rectangular-shaped pores on the surface of the valve and silicified struts on the edges (Fig. 1I). Pores were approximately 103-113 nm wide and varied in length between 140-240 nm (Fig.1I). Regularly repeating pore structures, such as seen in *N. pelliculosa* and *N. alba*, are more likely to give rise to photonic band gap properties, suggesting that these species would be useful to examine in this regard.

3. Absorbance and Fluorescence Characteristics.

Solid silica from each diatom species was examined for absorbance characteristics (Fig. 2A). Sample amounts were not normalized in these measurements, thus the absolute absorbance of each does not necessarily correlate to an inherent characteristic of silica from a given species. The general trend in absorbance was similar for all samples in that it increased gradually with decreasing wavelength (Fig. 2A). This is in contrast to the absorbance characteristics of pure glass, which does not change in the visible range, but increases dramatically below 300 nm. The underlying mechanism for the response of the diatom material is unclear, but perhaps the increasing absorbance with shorter wavelength in the visible range was due to the presence of organic material (i.e. peptides or polyamines) occluded within the silica (Kröger et al., 1999, 2000).

Diatom silica fluorescence when excited at 500 nm is shown in Fig 2B. The apparent fluorescence between 520-540 nm may be due to spill over from the excitation source. These data suggest that diatom silica exhibits little fluorescence at these wavelengths.

4. Dye Staining of Diatom Silica.

It had previously been shown that diatom silica could be stained *in vivo* by the fluorescent dye rhodamine 123 (R123), and that the dye was incorporated only into actively polymerizing silica (Li et al. 1989). We tested the ability of six other fluorescent dyes (Table 1) to stain diatom silica, using *N. pelliculosa* as a test organism. Cells were incubated with 2 μg/ml of dye for 24 hr under growth conditions, then harvested and resuspended in 90% methanol, and examined by fluorescence microscopy (excitation 460-500 nm, emission 510-560 nm). To summarize these results, all three rhodamine derivatives stained cell wall silica (Table1, Fig. 3). A significant and novel finding from this study was that rhodamine B and 6G apparently stained the entire wall, including previously polymerized silica, very intensely (Fig. 3). The emission wavelength maximum for these two derivatives is higher than for R123, offering the possibility of dual staining newly synthesized and mature walls. Also, because staining with B and 6G was much more intense than R123, this may improve the optical characteristics of the material. Regarding other dyes tested, although acridine orange and SYTO 18 stained the cells, they did not stain cell wall silica, and lucifer yellow and lysotracker blue did not stain at all (Table 1).

The above experiments were done on cell material extracted with methanol. We tested whether R123-stained *N. pelliculosa* silica retained the dye after the harsh acid treatment required to extract purified silica. The absorbance spectrum of stained and control materials

were identical, but the fluorescence spectra showed a peak at 531 nm only in the R123 stained material (Fig. 4 A and B). This indicated that the dye was stably maintained after extraction. In aqueous or ethanol solutions, the emission maximum for R123 is 525 nm; the red shift in the diatom silica sample could be due to a change in the polarity of the dye's environment. Specifically, this could be due to pairing of R123 with anions (Emaus, et al. 1986), since a net negative charge of silicate anions would accumulate during silica polymerization.

5. Photonic Band Gap Measurements.

Species such as *N. pelliculosa* and *N. alba* may be more likely that the others to have photonic band gap (PBG) properties due to the regular arrangement of their pores. An attempt to measure PBG properties was performed by Dr. Sean Kirkpatrick at AFRL. It was unsuccessful, due to scattering by the sample. One possible solution to this problem would be to develop a method to regularly orient the cells prior to measuring. This has not been attempted.

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Personnel Suported

Dr. Mark Hildebrand, Assoc. Proj. Scientist.

Publications

None.

New Discoveries

Diatom silica can be stained with rhodamine B and 6G.

Honors/Awards

None.

Table 1. Summary of Diatom Silica Dye-Staining Experiments

Dye Tested	Fluorescence*	Cell Wall Silica Staining
Rhodamine 123	Yes	Yes (actively polymerizing)
Rhodamine B	Yes	Yes (mature wall)
Rhodamine 6G	Yes	Yes (mature wall)
Acridine orange	Yes	No (nucleic acid stain)
Lucifer yellow	No	
Lysotracker Blue	No	
SYTO 18	Yes	No (mitochondrial stain)

* Fluorescence was measured on methanol-extracted samples under conditions described in the text.

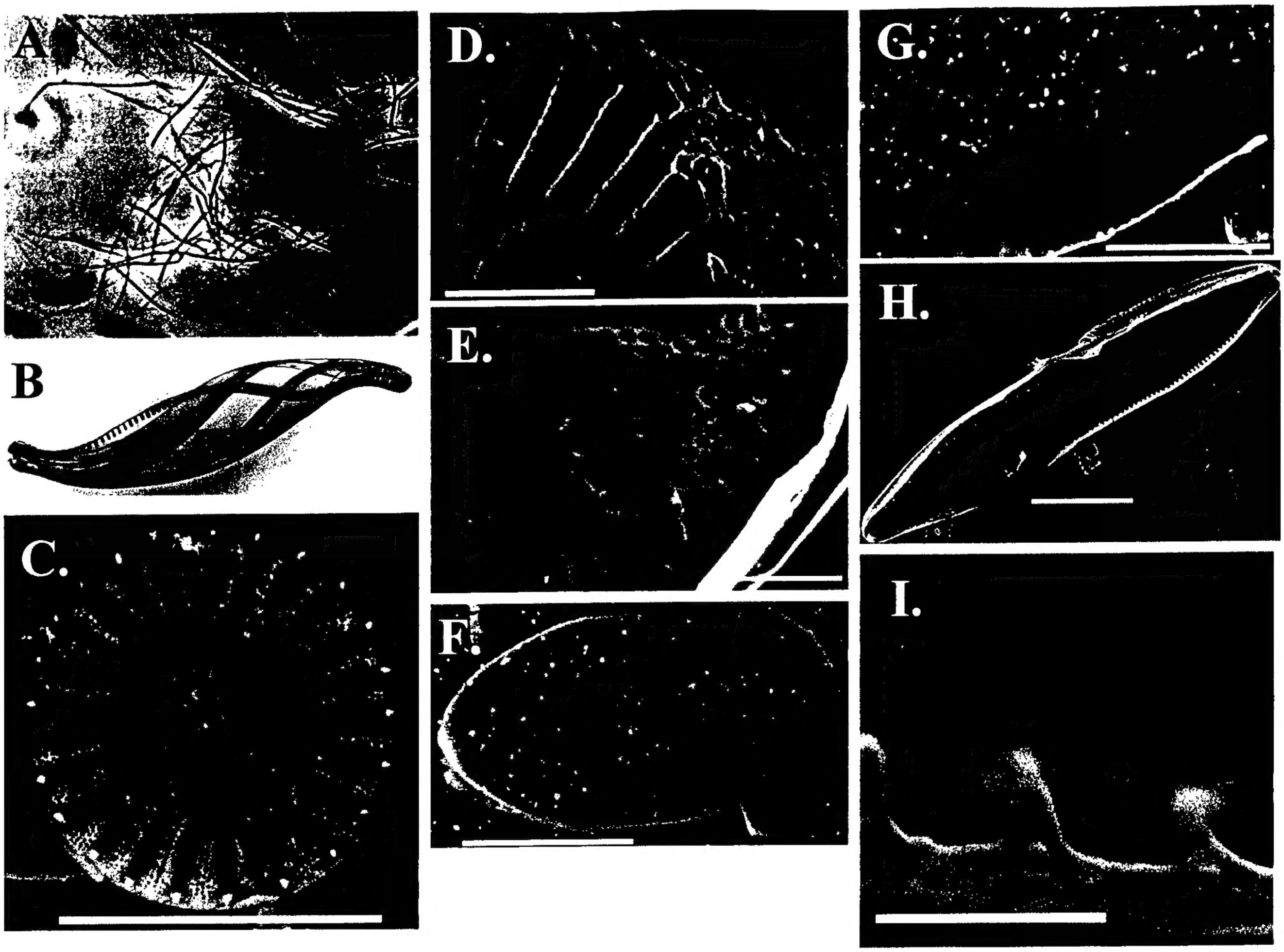
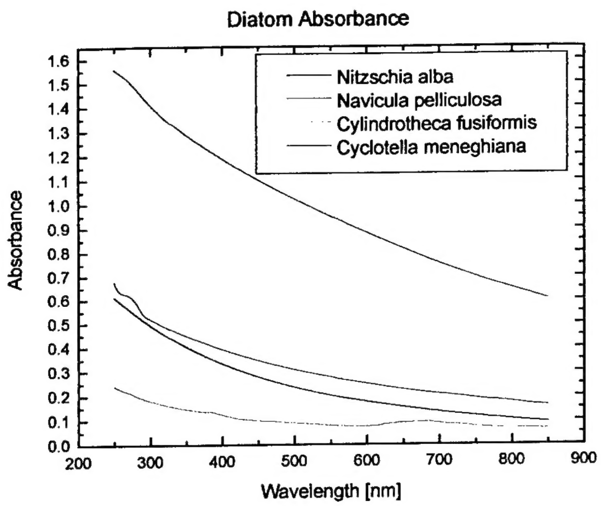


Figure 1. Images of diatom cell walls used in this study. **A.** Light micrograph of silicified cell wall material isolated from *Cylindrotheca fusiformis*. **B.** Artist's rendition of *C. fusiformis* (from Reimann, et al. 1965). **C.** Upper surface of *Cyclotella meneghiana*, scale bar 10 µm. **D.** View of edge of upper surface of *C. meneghiana* at an angle, showing the ridges and pores on the outer margin. Scale bar 2 µm. **E.** Irregular pores on the outer margin of the wall of *C. meneghiana.* Scale bar 1 µm. **F.** Upper surface of *Navicula pelliculosa*, scale bar 3 µm. **G.** Upper surface of another *N. pelliculosa* cell, scale bar 2 µm. **H.** Inner cell surface of *Nitzschia alba*, scale bar 5 µm. **I.** Junction of bottom and side walls of the inside of *N. alba*, scale bar 1 µm.



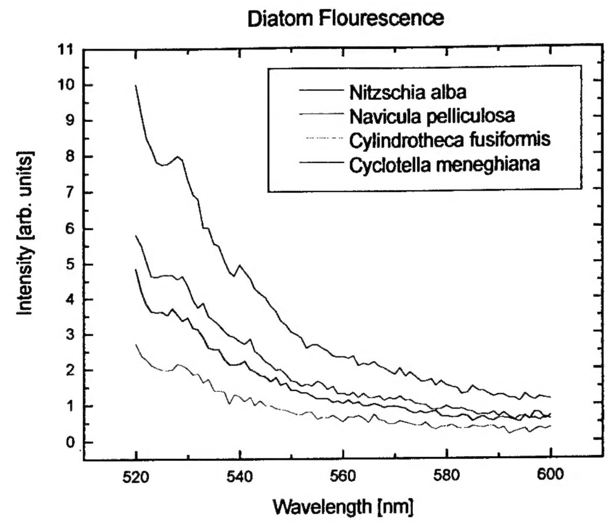


Figure 2. Absorbance and fluorescence spectra of diatom silica.

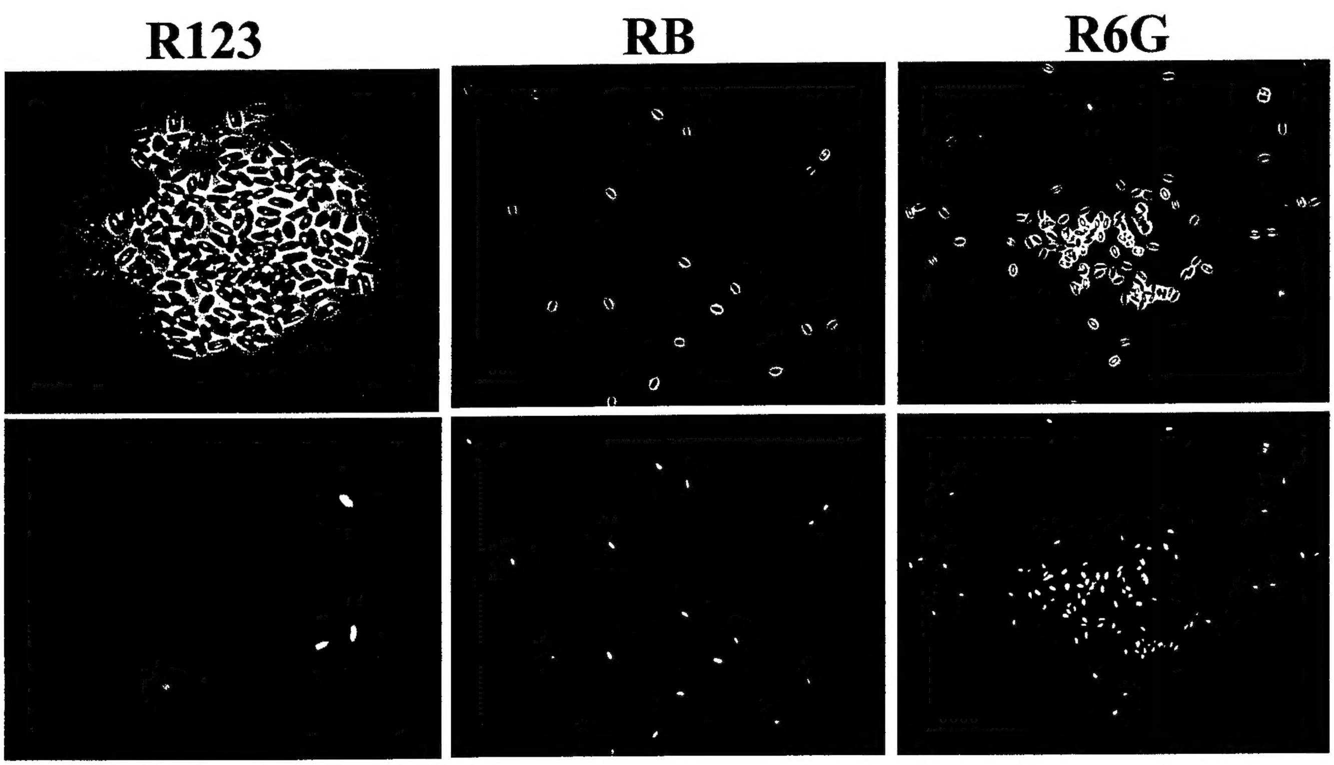


Figure 3. Staining of diatom cell wall silica with rhodamine 123 (R123), rhodamine B (RB), and rhodamine 6G (R6G). Upper panels are bright field images, and lower are fluorescence images.

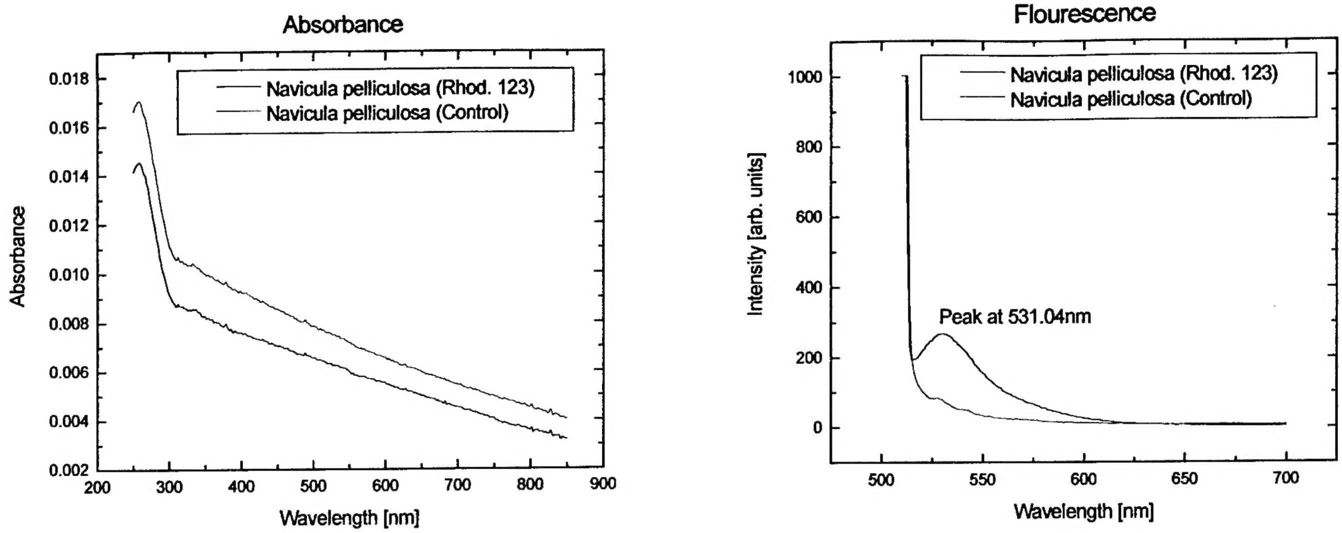


Figure 4. Comparison of absorbance and fluorescence spectra of Rhodamine 123 stained and unstained (control) silica isolated from *Navicula pelliculosa.*